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14. ABSTRACT
Fatty acid synthase (FASN), the enzyme that synthesizes fatty acid in cells, is over-expressed in prostate cancer and a potential therapeutic target. We have identified several novel chemical scaffolds with potential to inhibit FASN, from several hundred compounds discovered and synthesized by us. An extensive series of compounds with anti-FASN properties have been synthesized and characterized for their ability to inhibit recombinant FASN, FASN activity in tumor cells, and to kill prostate cancer cell lines. The best inhibitors have increased potency over other FASN inhibitors, including orlistat, the prototype FASN thioesterase inhibitor. There are solubility issues with some compounds, decreasing delivery and effectiveness. The low solubility is likely due to the hydrophobic nature of the TE domain, owing to the fact that is recognized saturated fat as a substrate. This report summarizes the the immense amount of structure-activity-relationships for new compounds we developed and reports on a new click-chemistry approach to derive novel FASN inhibitors.

15. SUBJECT TERMS- fatty acid synthase, thioesterase, inhibitors, drug development

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Introduction

The purpose of this proposal was to develop and optimize chemical scaffolds as potential inhibitors of fatty acid synthase (FASN), specifically the thioesterase (TE) domain. This line of investigation was based on a series of observation by many groups, including ours, that FASN represents a valuable drug target. It isoverexpressed in prostate cancer and appears to be required for tumor cells to survive. Through an iterative scheme of *in silico* design, activity-based screening and structural analyses we identified a series of novel pharmacophores with the ability to inhibit the thioesterase domain of FASN. This proposal had three specific aims. They were 1) To optimize compounds through structure-based design, chemical syntheses and *in vitro* testing, 2) To determine the toxicological and pharmacokinetic properties of the most promising analog(s), and 3) To test the efficacy of the analog(s) in mouse xenograft models of human prostate cancer. The proposal was successful in many ways, although an immediate candidate for translation to the clinic has not yet been identified. We identified several chemical scaffolds that have capacity to inhibit FASN TE; we identified a new method in click chemistry to generate novel FASN inhibitors; we also developed new synthetic strategies in the generation of the novel compounds we have described. Lastly, two of our postdoctoral trainees transitioned into tenure track faculty positions. The sections below will recap and summarize our major findings, borrowing some aspects from previous reports because that work is complete and manuscripts are pending and including new sections highlighting the latter portions of our studies.

Body

Our discovery, design and medicinal chemistry efforts have led to the synthesis of more than eighty (80) fully characterized compounds representing six structural classes: 5,6-quinoline-diones, naphthylene-1,4-diones, 1,4-benzoquinones, 1,4-hydroquinones, benzo[*d*]isoxazole-4,7-diones and 1*H*-indazole-4,7-diones. In addition, numerous precursors, numbering in the hundreds have also been generated. The novel members of these classes are the subject matter of three provisional patent applications. All salient data collected, thus far on these compounds is summarized in Appendix A. We have also developed a new methodology, targeted click-chemistry, for the derivation of novel classes of FASN inhibitors.

I. Pharmacological and *in vitro* data novel compounds

Based on our medicinal chemistry efforts and data collected thus far, the 1*H*-indazole-4,7-dione scaffold appears to be a flexible template for further optimization. Figure 1 summarizes several compounds we have selected for further optimization, and the data for 86 compounds is summarized in the appendix.

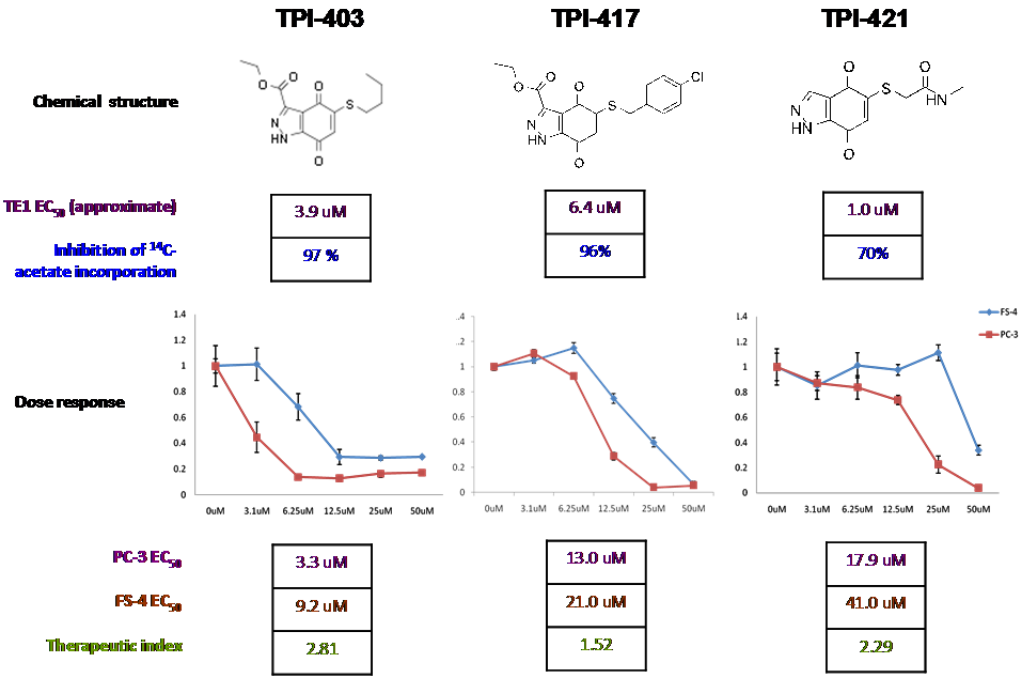
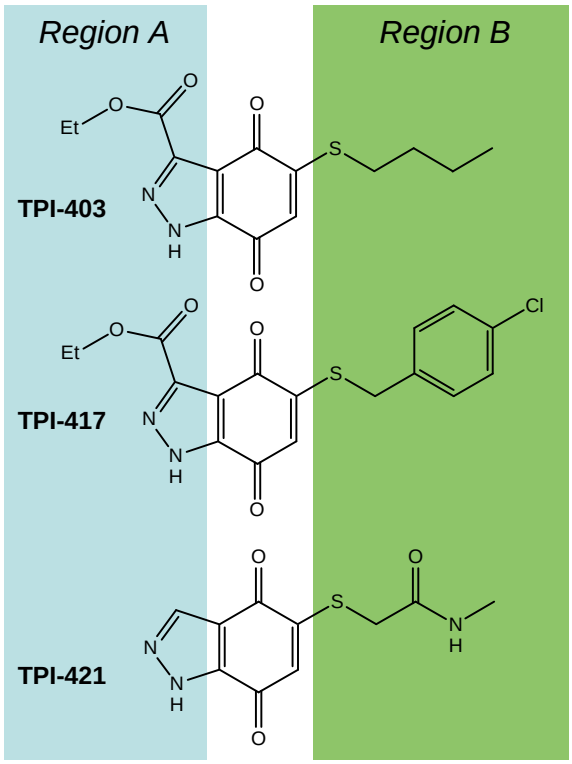


Figure 1- Lead Series Data Summary
Key: PC-3, prostate cancer cells; FS-4, normal fibroblast (control cell line); therapeutic index, {FS-4 EC_{50}}/{ FS-4 EC_{50}}

II. Further optimization strategy for TPI-403, TPI-417 and TPI-421

Further optimization of this series centers on two themes: (1) increasing affinity at TE and (2) increasing solubility in aqueous media. The former goal will also likely lead to a desired increase in therapeutic index of the series (defined here as $EC_{50(\text{normal cells})}/EC_{50(\text{cancer cells})}$).The structure-activity relationships thus far indicate that a wide variety of substituents are accommodated in Regions A and B of the 1*H*-indazole-4,7-diones scaffold. These regions are depicted in Figure 2.



The further optimization plan for Region A is summarized in Figure 3 and will take advantage of the fact that the 5 position of the 1,4-dihydroquinone intermediate (blue structure, Figure 3) is highly susceptible to nucelophilic attack. In addition, well-established Diels-Alder chemistry will be used to create additional fused ring structures (structures 3d and 3f). Other key targets include: the introduction of various substituents (R1) into the indazole ring of structure 3a; and coupling of various aldehydes and α,β-unsaturated ethers to the 5 position of the quinine under acidic conditions to yield compounds like 3c and 3e.

Figure 2- 1*H*-indazole-4,7-dione optimization regions

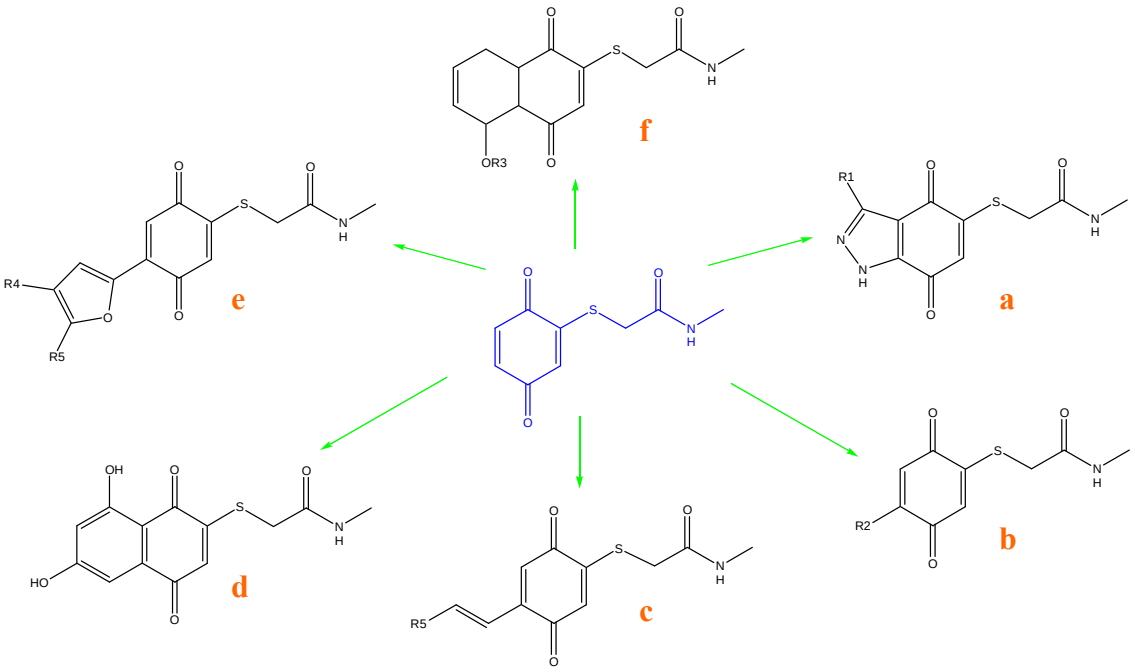


Figure 3- Region A optimization strategy for TPI-403, TPI-417 and TPI-421

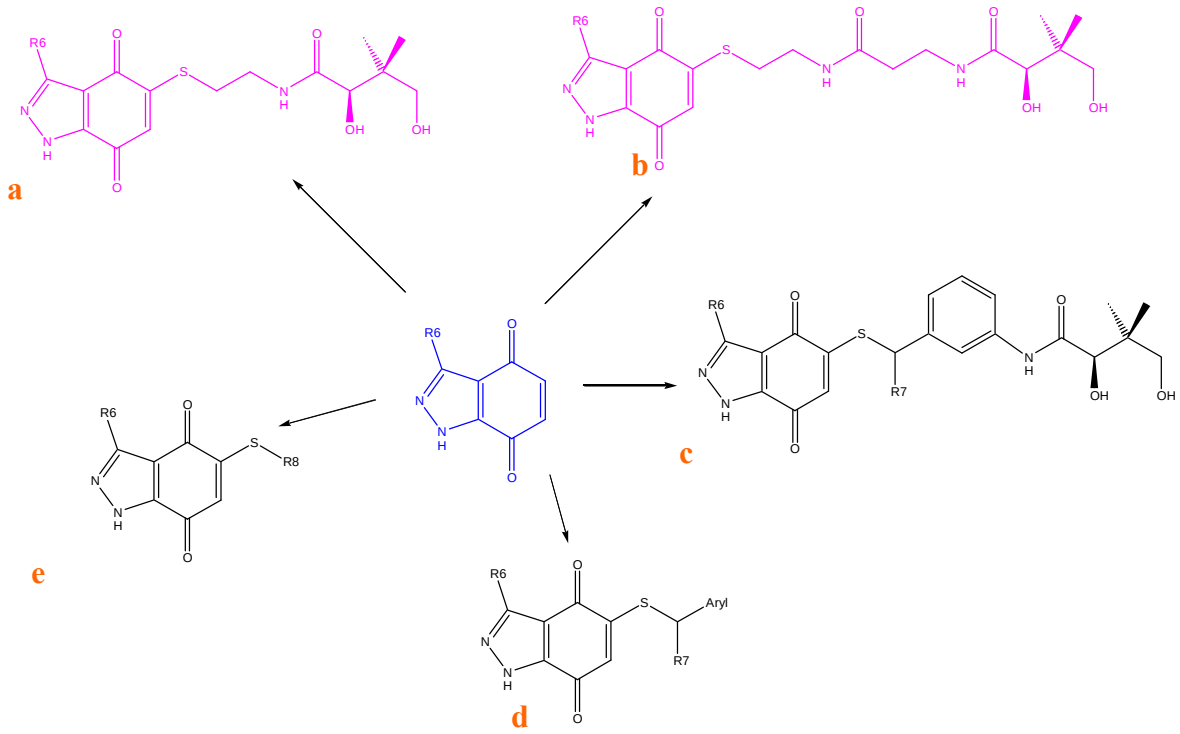


Figure 4- Region B optimization strategy for TPI-403, TPI-417 and TPI-421

The proposed further optimization of Region B, is shown in Figure 4. Here we will take advantage of crystallographic and docking data generated by our laboratories. Together these data demonstrate that

substituting a pantetheine moiety onto the 1*H*-indazole-4,7-diones position of the 1*H*-indazole-4,7-dione scaffold (blue structure, Figure 4) would preserve the likely binding mode of the quinone near the catalytic triad of TE while packing the pantetheine channel, which is a unique feature of TE. We surmise that the introduction of a pantetheine moiety in a favorable orientation will not only significantly increase TE affinity and solubility, but will also increase specificity of the series toward the target. Why? Because pantetheine is a cofactor used exclusively for fatty acid synthesis, which is an absolute requirement of epithelial cancer cells and is also known to correlate with tumor aggressiveness. Examples of pantetheine -like target compounds are shown in Figure 4: structures 4a and 4b; structure 4c is an analog of TPI-417 that attempts to preserve the aromatic moiety adjacent to the indazole ring, while introducing key features of pantetheine.

Backup Compounds and Other Findings

III. 5,6-quinoline-diones

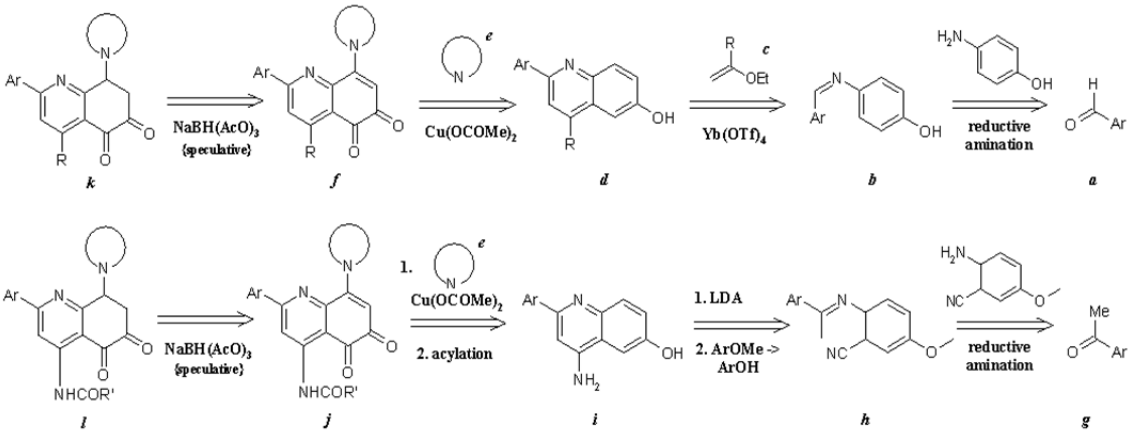


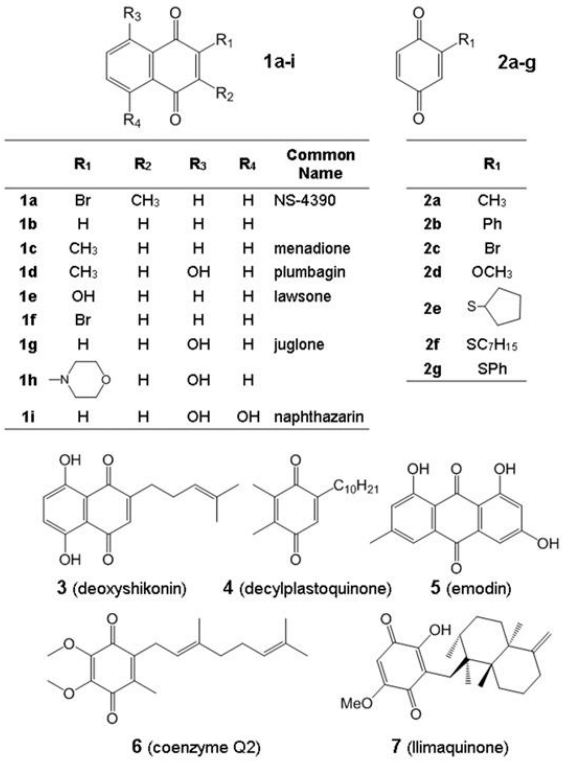
Figure 5- Synthetic strategy for 5,6-quinoline-diones

Based on our finding that the Nanosyn library compound containing the 5,6-quinoline-dione moiety (TPI-100, see Appendix A for structure) inhibits recombinant FASN TE and cancer cell growth, we pursued development of novel analogs of this 5,6-quinolinedione. Following the synthetic scheme shown in Figure 5 we were able to synthesize 10 5,6-quinoline-dione analogs. While structure-activity relationships indicated a clear trend towards a more optimal biological profile, we turned our attention toward the promising and easily synthesized 1,4-naphthoquinones and 1,4-benzoquinones.The chemistry of the 5,6-quinoline-diones have proven to be challenging due to low yields and lack of 'generalizability'. The overall synthesis up to the hydroxyquinoline stage (structure *d*) is efficient and gives high yields overall, but the critical oxidation step (*d* → *f*) provided only marginal yields and did not work with many of the amines (*e*) of interest.

IV. 1,4-naphthoquinones and 1,4-benzoquinones

An analysis of naturally occurring, commercially available, and new synthetic 1,4-benzoquinones, 1,4-naphthoquinones, 9,10-anthraquinones (Figure **6** and Chart 1) was performed to develop SAR information for FASN-TE at an initial concentration of 10 μM. Included are the naturally occurring compounds menadione (**1c**), plumbagin (**1d**), lawsone (**1e**), juglone (**1g**) and napthazarin (**1i**). The 1,4-naphthoquinones (**1a-i**) and 1,4-benzoquinones (**2a-g**) (Table 1) display potent inhibition of FASN-TE, except for compound **1e**. In contrast, the more complex naturally occurring compounds (**3-7**) inhibit with less potency (Table 1). The 1,4-quinones with known anti-cancer activity like mitomycin C, doxorubicin, geldanamycin, streptonigrin and mitoxantone did not inhibit; lapachol and β-lapachone exhibited weak inhibition potency (data not shown). All of the 1,4-benzoquinones (**2a-c**,**2e-g**) with the exception of **2d** show complete inhibition (**Table 1**) of FASN-TE at 10 μM, but because of their high level of chemical reactivity were not assessed further with TE or in cellular assays.

The IC_{50} values against recombinant TE were determined for compounds **1a-i**, **2e-g**, and **3**. The 1,4-naphthylquinones (**1a-i**) show a much wider SAR profile with the IC_{50} values ranging from 0.1 μM to greater than 100 μM. As expected, compounds **1b**, **1f** and **1g** had the lowest IC_{50} values of 0.9 μM, 0.3 μM and 0.1 μM, respectively, and compound **1e** had the highest IC_{50} value of over 100 μM. Based on this dataset, we reasoned that the structure-activity is driven principally by substituent effects of moieties directly attached to the quinone ring. Overall activating groups (Electron Donating Groups, EDGs) diminish inhibition, while deactivating groups (Electron Withdrawing Groups, EWGs) have nominal to a positive effect on inhibition. Compound **1e** containing a strong EDG consistent with the SAR has low inhibition capacity; likewise compound **4** with three weak EDGs is a nominal inhibitor. The natural products **6** and **7** that contain strong EDGs (methoxy and hydroxy) also show diminished ability to inhibit TE.



	R_1	R_2	R_3	R_4	Common Name
1a	Br	CH_3	H	H	NS-4390
1b	H	H	H	H	
1c	CH_3	H	H	H	menadione
1d	CH_3	H	OH	H	plumbagin
1e	OH	H	H	H	lawsone
1f	Br	H	H	H	
1g	H	H	OH	H	juglone
1h		H	OH	H	
1i	H	H	OH	OH	naphthazarin

	R_1
2a	CH_3
2b	Ph
2c	Br
2d	OCH_3
2e	
2f	SC_7H_{15}
2g	SPh

Figure 6. 1,4- and 9,10-diones tested against recombinant FASN-TE and FASN

To test this hypothesis and to reduce overall chemical reactivity, several analogs were synthesized: one compound with a strong EDG (**1h**) resulting from the treatment of juglone with morpholine and some with weak to very weak electron donating groups (EDGs; **2e-g**). As shown in Chart 1, these compounds were synthesized by reaction of the quinone precursor with a sulfur or nitrogen nucleophile leading to the substituted 1,4-dihydroxy compound (the reduced form of the quinone). Oxidation of the thiol intermediates was accomplished in high yield by dissolving the thiol substrates in methylene chloride and using an aqueous solution of sodium periodate and benzyltrimethylammonium bromide as a phase transfer catalyst[13] to afford the desired product (**2e-g**). In contrast, the morpholino containing intermediate auto-oxidized to provide the desired compound (**1h**).

Supporting our hypothesis, the thio compounds (**2e-g**) are potent inhibitors; while the morpholino compound (**1h**) was a nominal inhibitor. The improved inhibition of the former compounds is most likely due to their lack of aromatic rings around the 1,4-benzoquinone core structure, as the naphthoquinone **1h** or the anthraquinone **5** do, and the missing electron cloud of the phenyl rings decreases the electron donating capability of the 2-alkyl-substituted 1,4-benzoquinone derivatives. We surmise that Michael addition is one plausible mechanism for enzyme inhibition, though analogs with fully substituted quinone rings (**1a**, **5**, **6**, **7**) still retain the ability to inhibit. Further work will be required to develop a mechanistic basis for the observed SAR; for example, affinity measurements (K_i) and determination of reversibility of inhibition will be critical in uncovering the physicochemical determinants of the observed inhibition data.

Inhibition of cellular FASN activity was performed for selected compounds in two prostate cancer cell lines (PC3 and DU145) and in normal fibroblast (FS4) using an assay that measures the incorporation of the ^{14}C-acetate precursor into lipids.[14] Not surprisingly, there is little correlation between the ability of a compound to inhibit recombinant TE and inhibition of cellular FASN activity (Pearson's R=0.1). Interestingly, the compounds that best inhibited ^{14}C-acetate incorporation are in the simple 1,4-naphthoquinone family (**1a, 1b**, **1d**, **1f**, **1g**, **1i**). The most potent inhibitors of recombinant TE that showed no inhibition of endogenous enzyme

are the 2-thio-substituted 1,4-benzoquinones (**2e-g**). A high level of nonspecific chemical reactivity and/or a myriad other factors may be contributing to the observed contrast in SAR between these assays.

There is also a correlation between the ability of a quinone analog to inhibit cellular FASN and the potency (EC_{50}) at killing tumor cells. The inhibition of ^{14}C-acetate uptake and EC_{50} for inhibition of PC3 cell growth is correlated with a Pearson's r=0.85. Prostate cancer cells expressing the highest level of FASN (DU145) used in this study show an even higher correlation, r=0.92. Despite this strong relationship, only a few of the compounds demonstrated a marginal therapeutic index which was calculated as the cell killing ratio of cancer and FS4 fibroblasts EC_{50} values (data not shown).

TABLE 1. Effect of 1,4- and 9,10-diones on FASN-TE, fatty acid synthesis and cell killing

Cmpd	rTE[a] % Inhibition at 10 µM	rTE IC_{50} (µM)	Inhibition of FA Synthesis IC_{50} (µM)	Cell Killing EC_{50} (µM)		
			PC-3	FS4	PC-3	DU145
1a	78	12.5	10	9.4	11.8	19.2
1b	100	0.9	2.8	10.6	12.8	6.8
1c	54	21.9	–[b]	16.7	48.4	–[b]
1d	59	10.9	2.4	2.3	4.1	5.3
1e	10	>100	–[b]	>50	>100	–[b]
1f	100	0.3	11.5	21	23.3	35.4
1g	100	0.1	6.8	6.3	5.8	10.6
1h	26	26.4	–	–[b]	–[b]	–[b]
1i	98	3.5	2.6	2.4	2.7	4.8
2a	100	–[b]	–[b]	–[b]	–[b]	–[b]
2b	100	–[b]	–[b]	–[b]	–[b]	–[b]
2c	100	–[b]	–[b]	–[b]	–[b]	–[b]
2d	66	–[b]	–[b]	–[b]	–[b]	–[b]
2e	99	6.4	> 25 µM	–[b]	–[b]	–[b]
2f	96	6.2	>25 µM	–[b]	–[b]	–[b]
2g	100	0.2	>25 µM	–[b]	–[b]	–[b]
3	4	52.4	>25 µM	31.1	8.3	–[b]
4	39	–[b]	–[b]	–[b]	–[b]	–[b]
5	35	–[b]	–[b]	–[b]	–[b]	–[b]
6	30	–[b]	–[b]	–[b]	–[b]	–[b]
7	31	–[b]	–[b]	–[b]	–[b]	–[b]

In summary, we have demonstrated that 1,4-benzoquinones and 1,4-naphthoquinones, as well as a number of natural products containing the 1,4-benzoquinone and the 9,10-anthraquinone moiety inhibit the TE domain of FASN as well as cellular FASN activity. Additionally, the SAR shown herein is a starting-point for the rational design of therapeutics that target the TE domain of FASN. A manuscript describing this data is currently under review.

Enhancing discovery horsepower through Click chemistry (CC).

The costs and complexity of drug discovery present a barrier-to-entry for many academic researchers who frequently possess otherwise highly drug-able targets. In this proposal we address one aspect of this problem: the costly and time-consuming process of conventional medicinal chemistry. The inspiration behind

our efforts to accelerate our FASN Drug Development Program (FASDDP) comes from the broad field of target-guided synthesis, originally described by Rideout and coworkers (1,2). Target-guided synthesis offers an attractive alternative to traditional lead optimization techniques. By making use of a protein target as a nanoscale reaction vessel, only the building blocks that fit into the confines of the protein binding site(s) can react to form new compounds. In a recent extension of this methodology known as *in situ* Click chemistry (CC), Rostovtsev and coworkers use the bioorthogonal Huisgen cycloaddition reaction to identify novel high affinity ligands (3). They and other investigators demonstrated that very high-affinity compounds can be identified with relatively little effort (4-6). In a CC experiment, a set of alkynes and azides are combined with target protein in aqueous buffer under ambient conditions. Those alkynes and azides that bind with an orientation favorable to cycloaddition form new triazole compounds. For example, numerous classes of acetyl-cholinesterase inhibitors have been developed, many with femtomolar binding affinities (7). This body of literature also confirms that the free-solution Huisgen reaction is so slow (by a factor of 10^5) that false-positives are practically nonexistent. Finally, CC is considerably more efficient in exploring molecular diversity than conventional medicinal chemistry approaches. Filling the discovery pipeline with diverse leads is one of the most significant strategies for success in the drug discovery and development process.

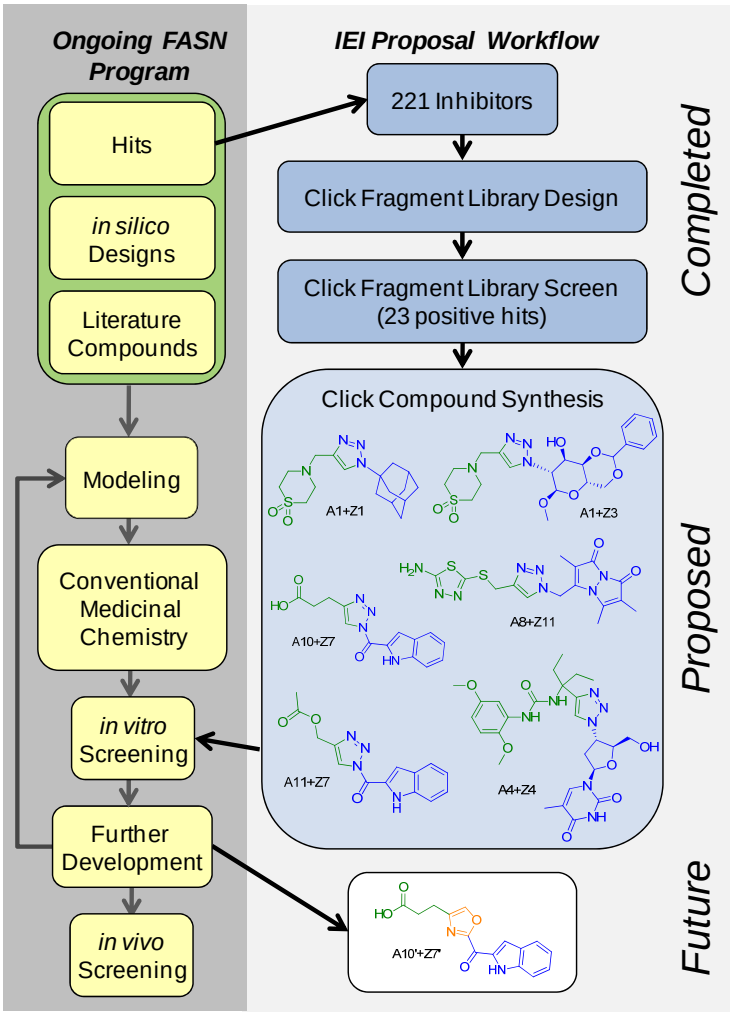


Figure 7. Proposed Click chemistry workflow to develop FASN inhibitors. The left column represents a traditional ongoing FASN drug discovery program. The right column is the proposed work flow for the proposed Click chemistry strategy. *Completed*: The generation of 23 potential Click leads against FASN has been performed. *Proposed*: The 6 Click-derived compounds that will be synthesized and screened for the ability to inhibit FASN. *Future*: A modified version of the A10-Z7 lead where the triazole ring is replaced with a different moiety.

We first discovered that the FDA-approved drug Orlistat can inhibit FASN, selectively kill tumor cells and inhibit the growth in prostate tumor xenograft in mice (8). Specifically, Orlistat inhibits the thioesterase (TE) domain of FASN, the terminal step of fatty acid synthesis. We subsequently solved the first crystal structure of FASN-TE bound to Orlistat (9). This structure revealed that FASN-TE contains three distinct binding pockets. The specificity or hydrophobic channel binds the growing fatty acid chain and guides substrate specificity of the enzyme. The short-chain pocket contains the active site serine of the enzyme. Lastly, the pantetheine channel interacts with the acylated acyl-carrier protein of FASN. These results, combined with previous studies, highlight the broad potential of FASN as a therapeutic target and suggest multiple strategies to block enzyme activity. Moreover, one could envision that the three unique binding pockets provide multiple environments to accommodate Click fragments in novel conformations.

Although Orlistat is an FDA-approved drug, it has several shortcomings that limit its potential as an anti-cancer therapeutic. The driving factor is that Orlistat does not reach systemic circulation. Rather it is active in the gut and that which reaches circulation is rapidly inactivated. Based on these facts, a combined activity- and structure-based chemical library screening strategy was devised to identify novel chemical scaffolds with

potential to inhibit FASN-TE (**Fig. 7, left column**). This strategy follows the traditional drug discovery paradigm. Using a library of 8,800 compounds, a subset of 221 hits was identified as potential FASN inhibitors. In an effort to increase the novelty and diversity of the chemical scaffolds with FASN-inhibitory potential, a CC approach was devised (**Fig. 7, right column**). We took advantage of the existing data in hand and generated a library of 19 alkynes (A fragments) and 11 azides (Z fragments). Each A and Z fragment was selected based their enriched occurrence in the initial pool of 221 FASN leads. The A-Z pairings represent a focused library of 209 combinations. In addition to the presence in the initial screen, the 19 A fragment and 11 Z fragments were also vetted against patent art for "uniqueness" as anti-cancer compounds. To generate the Click leads, each A-Z pair was incubated overnight with recombinant FASN-TE. If an A and Z fragment bind within the FASN-TE active site with the correct special orientation, the azide and alkyne moieties will spontaneously react to form the Click compound. After the overnight reaction at room temperature, the reaction was stopped and enzyme was removed by precipitation. The formation of Click compounds was determined by screening for the unique mass signature of each theoretical compound by mass spectrometry. The experiment yielded 23 unique Click compounds, a high proportion consistent with the focused nature and design of the A/Z fragment library. These are represented in the "*Completed*" portion of **Figure 7**. From these 23, we plan to synthesize 6 compounds based on: 1) availability of reagents, 2) ease of predicted synthetic strategy, and 3) prediction of FASN inhibitory potential. The A fragments are highlighted in green and the Z fragments are highlighted in blue. This is represented in the "*Proposed"* portion of **Figure 7**. The "*Future*" portion of the figure shows a possible core modification that may be pursued. We anticipate that core modification will be necessary to optimize the chemical functional groups further and to define a core that is clear of any potential patent space or intellectual property issues

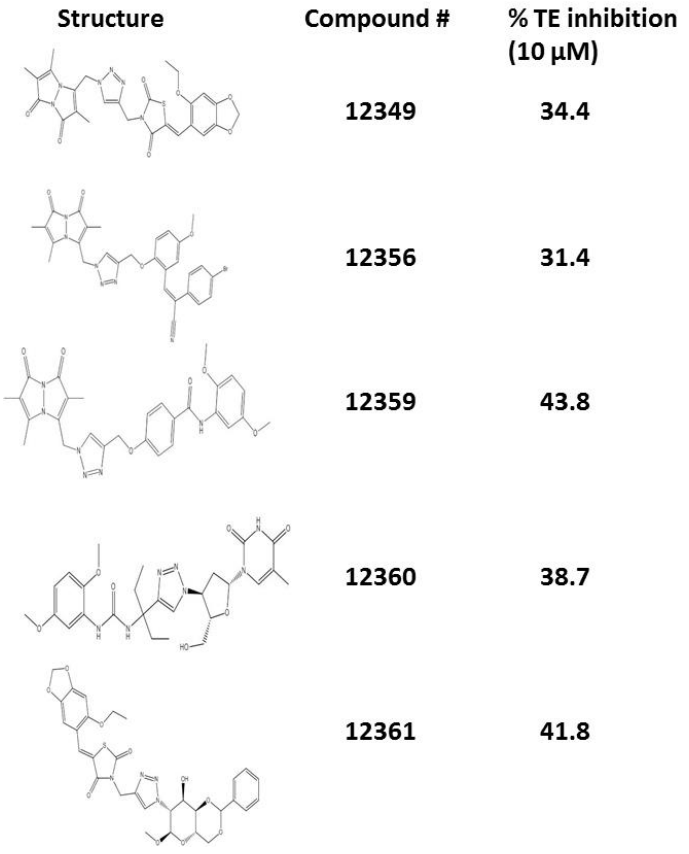


Figure 8. Click-chemistry derived FASN-TE inhibitors.

Synthesis of Click leads. We identified 23 leads and had them synthesized for subsequent evaluation (**Fig. 8**). From the initial 23 Click hits, 5 azide and alkyne fragment combinations demonstrated some FASN-TE inhibitory capacity. For example, at 10 μM compound 12349 inhibited about 34% of TE activity, 12356 inhibited 31%, 12359 inhibited almost 44%, 12360 almost 39% and 12361 almost 42%. This illustrates that the Ki for these compounds will be well above what our previous compounds demonstrated, but it the realm of being able to be modified to improve inhibition. The other compounds demonstrated no appreciable TE inhibition (not shown).

There remain several significant points to be made about this strategy. First, to our knowledge, this strategy is unique for the identification of FASN inhibitors. In addition, all of the compounds put into the screen were pre-vetted for their novelty in terms of intellectual property. Should useful compounds be derived, they will certainly have IP potential as well as clinical utility. Therefore, there remains a change for further development and translation of the compounds into the clinic.

Key Research Accomplishments:

- Synthesis and characterization of more than 80 novel FASN inhibitor scaffolds. (see Appendix)
- Optimization of FASN inhibitors of novel chemotypes
- Development of new synthetic strategies via click chemistry and 5 resulting novel compounds that can inhibit FASN TE.

Reportable Outcomes:

Manuscripts

1. DeFord-Watts, L.M., Mintz, A. and **Kridel, S.J**., The Potential of ^{11}C-acetate PET for Monitoring the Fatty Acid Synthesis Pathway in Tumors (2010) *Current Pharmaceutical Biotechnology, In press*
2. Odens, H.H., **Kridel, S.J**., Lowther, W.T., Watts, L.M., Filipponi, L.E., and Schmitt, J.D., Inhibition of the Thioesterase Activity of Human Fatty Acid Synthase by 1,4-Quinones. *In preparation*
3. **Kridel, S.J.**, Johnson, L., Wheeler, F., Filipponi, L.E., Odens, H.H., Schmitt, J.D., and Lowther, W.T., Structure Activity Relationships of Novel naphthoquinones that target the Fatty Acid Synthase Thioesterase Domain. *In preparation*.

Funding received, based on this award

1. SPARK Grant (Innovation and Entrepreneurship Initiative) 10/01/11-9/30/12
   Kridel (PI) $35,000
   Development of Novel Fatty Acid Synthase Inhibitors through Targeted Click Chemistry
   The goal of this project is to use Click-chemistry to evolve new FASN inhibitors from enriched fatty acid synthase fragments that have previously been identified.

   This intramural grant is an extension of the work from this LCT award and will provided a small pool of support to pursue some novel click-chemistry methodologies to derive novel FASN inhibitors from our previously determine pool of validated FASN inhibitors.

2. R01 CA161503 NIH/NCI 07/01/12-04/31/17
   Kridel, PI (4.2 months) $227,074
   NAD^+ metabolism in prostate cancer
   The goal of this project is to determine the role of NAD+ metabolism in prostate cancer. Specific emphasis will be placed on understanding the integration of Nampt, the sirtuins and CD38 in the regulation of lipid metabolism and survival of prostate tumor cells.

   This NCI funded award is not directly related to the DOD sponsored LCT award, but some of the work from the LCT award resulted in preliminary data that helped the application receive a fundable score.

Transition of trainees to faculty positions

1. Herman H. Odens, PhD: Dr. Odens was hired as a postdoctoral fellow to perform synthetic and medicinal chemistry and was promoted to Instructor in the Department of Biochemistry. Dr. Odens was recently hired as an Associate Professor in the Department of Chemistry at Southern Adventist University in Collegedale, Tennessee.

2. Laura M. Watts, PhD: Dr. Watts was hired as a postdoctoral fellow to characterize novel fatty acid synthase inhibitors in several cancer models. At the conclusion of her training, Dr. Watts was hired as Assistant Professor in the Department of Biology at Salem College in Winston-Salem, North Carolina.

Conclusion

As detailed in the body of this final report, we have synthesized a significant library of potential FASN inhibitors and expanded the potential repertoire of FASN inhibitors through a novel click-chemistry approach. These results highlight the significant effort that has been put forth as well as the hurdles that have been overcome. The implications of our finding are significant on multiple levels. First, the resulting data provides significant structure-activity-relationship (SAR) information around the active site of the TE domain of FASN. Because the FASN-TE domain of FASN is increasingly recognized as a potential therapeutic target in cancer, this information could make significant contribution to the development of FASN TE inhibitors, by our group or another. Second, the chemical scaffolds could provide the building blocks from which imaging probes could be derived. Third, and perhaps most important, is that we have identified a novel mechanism by which to identify new FASN inhibitors, that is click chemistry. In using the FASN-TE active site as the reaction vessel we have demonstrated the possibility that highly optimized inhibitors could be generated using that natural chemistry of the active site. The continued development of the click-derived compounds may elucidate not only new potential inhibitors, but also strategies to further optimize them.

It is clear from our studies, as well as those from others, that the development of FASN inhibitors for translation into the clinic will not be an easy task. The TE domain, while attractive as a target, may be hampered by its association with the serine hydrolase family of proteins. The expansiveness of this family may increase potential for reduced specificity. We are taking approaches to mitigate this such as improving solubility through different formulations, targeting through nanoparticles, and further compound optimization.

So what does this body of knowledge contribute? Several academic laboratories along with large and small pharma companies have or are currently developing inhibitors against FASN. In fact, the first FASN inhibitor is currently in clinical trials, having been developed by 3V Biosciences. For that matter, targeting metabolic enzymes is becoming a more attractive strategy in many types of cancers. The work presented in this report highlight design and optimization of novel FASN inhibitors. The results of this work will contribute to the development of FAS inhibitors and provide an avenue toward the translation of FAS inhibitors into the clinic for potential use in treating men with prostate cancer. It will do so through the identification of new chemical scaffolds that can target FASN and through the description of new click chemistry methodologies that utilize FASN as a chemical reaction vessel. Although a FASN inhibitor is currently being evaluated by clinical trial, there is ample opportunity to translate our findings into the clinic. The data from this proposal will contribute toward this goal.

References

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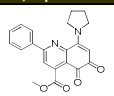
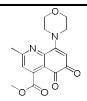
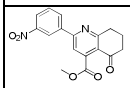
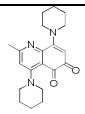
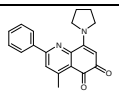
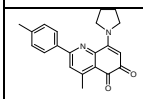
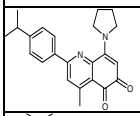
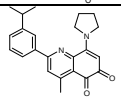
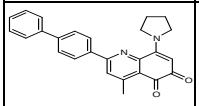
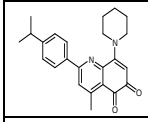
Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
5,6-quinolinediones										
	TPI-00100-00-A (NS-1456)	20.21	22.87	NA	NA	89.3	2.36	2.93	4.2	**1.78**
	TPI-00101-00-A	2.64	8.83	NA	NA	ND	6.4	6.36	ND	ND
	TPI-00102-00-A	17.27	14.45	NA	NA	ND	ND	ND	ND	ND
	TPI-00103-00-A	1.59	11.39	NA	NA	ND	>10	>10	ND	ND
	TPI-00104-00-A	0.00	12.04	NA	NA	ND	ND	ND	ND	ND
	TPI-00105-00-A	9.20	17.59	NA	NA	34	> 25	> 25	ND	ND
	TPI-00106-00-A	37.72	42.72	NA	NA	31	> 25	20.1	16.7	ND
	TPI-00107-00-A	51.68	54.24	11.59	13.78	49	18.2	14.33	9.4	0.52
	TPI-00108-00-A	21.26	26.36	NA	NA	23	> 25	> 25	ND	ND
	TPI-00109-00-A	30.25	26.80	NA	NA	7	> 25 uM	> 25 uM	ND	ND
	TPI-00110-00-A	28.01	46.00	NA	NA	ND	ND	ND	ND	ND
1,4-benzoquinones & 1,4-hydroquinones										
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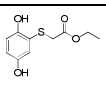
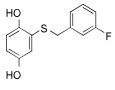
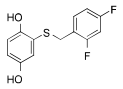
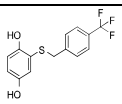
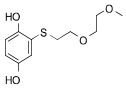
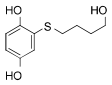
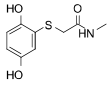
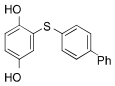
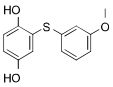
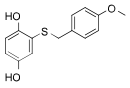
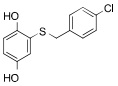
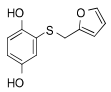
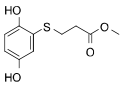
	TPI-00601-00-A	94.42	96.05	7.05	NA	65.7	32.2	>50	20.8	0.65
	TPI-00605-00-A	30.62	46.98	NA	NA	26.7	ND	ND	ND	ND

Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
	TPI-00606-00-A	10.10	14.34	NA	NA	20.3	ND	ND	ND	ND
	TPI-00607-00-A	45.23	19.65	NA	NA	34.3	ND	ND	ND	ND
	TPI-00608-00-A	35.37	93.22	NA	NA	86.2	22.4	>50	>50	ND
	TPI-00609-00-A	78.46	96.16	NA	NA	89.6	23	>50	32.4	**1.41**
	TPI-00611-00-A	31.33	8.42	NA	NA	24.5	ND	ND	ND	ND
	TPI-00612-00-A	0.00	47.84	NA	NA	6.25	ND	ND	ND	ND
	TPI-00613-00-A	13.21	70.02	NA	NA	18.9	ND	ND	ND	ND
	TPI-00614-00-A	37.05	89.56	NA	NA	35.2	ND	ND	ND	ND
	TPI-00615-00-A	18.19	NA	NA	NA	44.6	ND	ND	ND	ND
	TPI-00616-00-A	95.72	95.19	NA	NA	46.7	ND	ND	ND	ND
	TPI-00618-00-A	22.84	90.89	NA	NA	63.6	ND	ND	ND	ND
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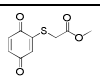
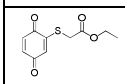
	TPI-00602-00-A	100.00	100.00	1.19	NA	33.3	37.3	>50	40	**1.07**
	TPI-00603-00-A	100.00	100.00	1.51	NA	16	>50	>50	>50	ND
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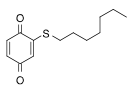
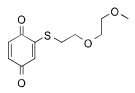
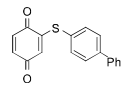
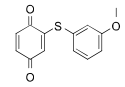
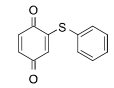
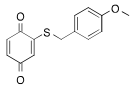
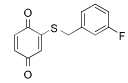
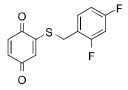
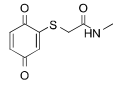
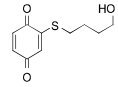
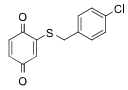
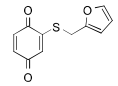
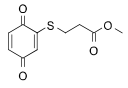
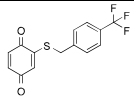
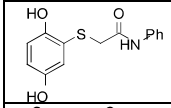
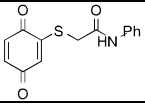
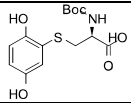
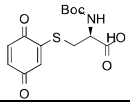
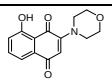
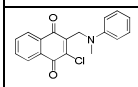
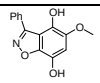
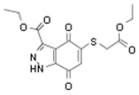
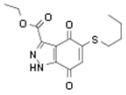
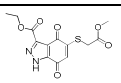
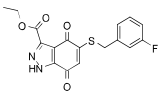
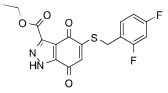
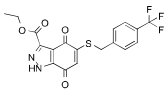
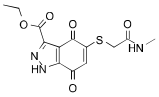
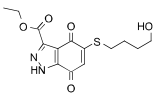
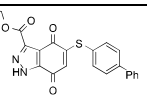
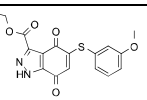
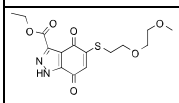
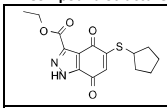
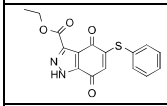
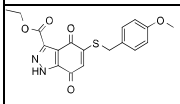
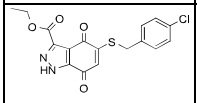
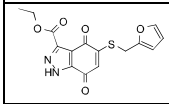
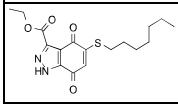
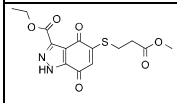
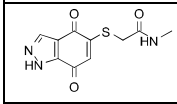
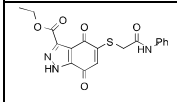
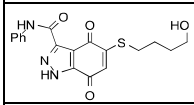
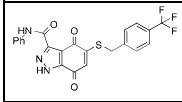
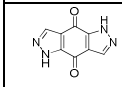
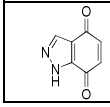
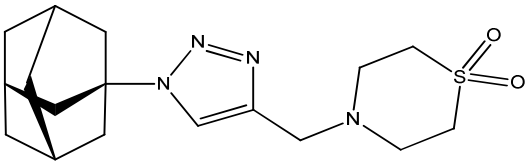
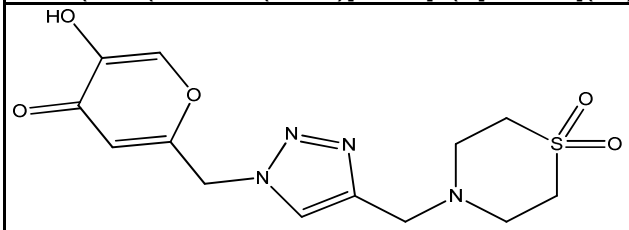
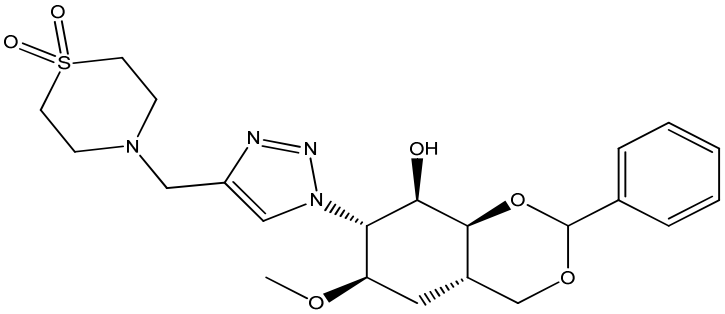
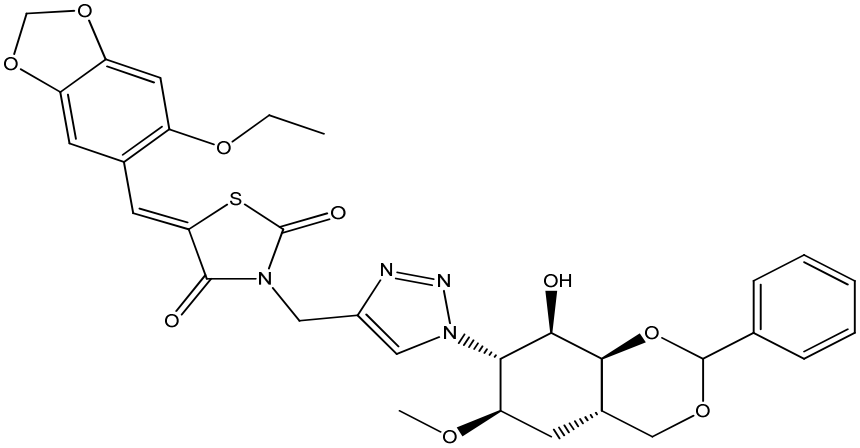
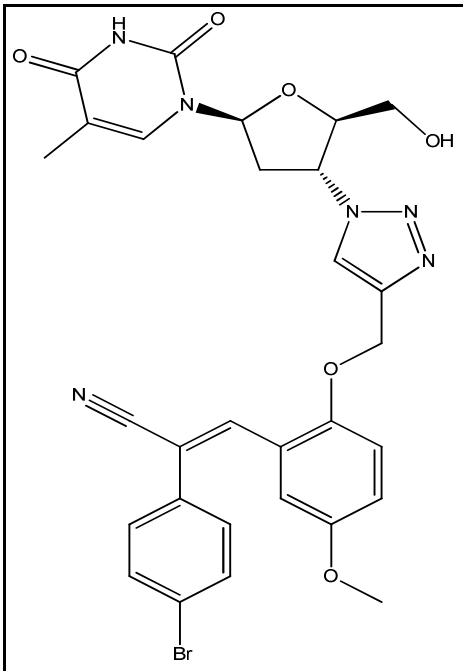
	TPI-00617-00-A	99.24	98.94	6.24	0.36	45	ND	ND	ND	ND

Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	recombinant thioesterase IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
	TPI-00620-00-A	98.13	98.88	1.70	0.12	91.4	20.8	ND	41.7	**2.00**
	TPI-00621-00-A	100.00	100.00	1.06	0.17	23.4	ND	ND	ND	ND
	TPI-00622-00-A	100.00	100.00	0.70	0.18	11.4	ND	ND	ND	ND
	TPI-00623-00-A	100.00	100.00	1.16	0.20	9.25	ND	ND	ND	ND
	TPI-00624-00-A	100.00	100.00	1.34	0.42	57.7	33	ND	20	0.61
	TPI-00625-00-A	100.00	100.00	1.10	0.25	64.3	22	ND	40	**1.82**
	TPI-00626-00-A	100.00	99.70	1.09	0.40	42.2	ND	ND	ND	ND
	TPI-00627-00-A	100.00	99.49	1.08	0.10	66.4	19	ND	37.5	**1.97**
	TPI-00628-00-A	100.00	97.81	1.16	0.23	53.7	>50	ND	>50	ND
	TPI-00629-00-A	100.00	100.00	1.08	0.24	50	ND	ND	ND	ND
	TPI-00630-00-A	100.00	100.00	1.55	0.34	61.85	ND	ND	ND	ND
	TPI-00631-00-A	100.00	100.00	1.44	0.11	91.9	>50	ND	42	ND
	TPI-00632-00-A	100.00	100.00	1.35	0.36	49.05	ND	ND	ND	ND
	TPI-00633-00-A	76.13	98.99	NA	NA	94.2	24.3	ND	38	**1.56**
	TPI-00634-00-A	100.00	100.00	NA	NA	69.7	24	ND	38	**1.58**
	TPI-00635-00-A	28.21	9.88	NA	NA	0	>50	ND	>50	ND
	TPI-00636-00-A	81.39	100.00	NA	NA	0	>50	ND	>50	ND

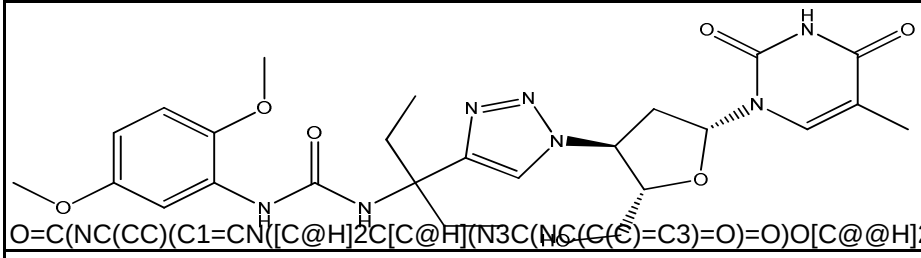
Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
naphthylene-1,4-diones, benzo[d]isoxazole-4,7-diones & 1H-indazole-4,7-diones										
	juglone	100.00	100.00	0.09	0.07	95.6	6.4	8.7	5.49	0.86
	TPI-00404-00-A	29.00	43.00	NA	NA	ND	ND	ND	ND	ND
	TPI-00400-00-A (NS-4390)	22.48	61.70	NA	NA	ND	29	>25	25.29	0.87
	TPI-00500-01-C (NS-4393)	100.00	100.00	1.08	0.41		18.75	19.2	ND	ND
	TPI-00501-01-A	44.28	34.75	NA	NA		ND	ND	ND	ND
	TPI-00401-00-A	40.00	55.88	NA	NA	29.1	33	>50	ND	ND
	TPI-00402-00-A	66.63	95.73	2.35	0.56	90.4	18.75	40	20.9	**1.11**
	TPI-00403-00-A	69.35	79.27	3.90	2.42	97 (IC_{50} = 6.75 μM)	3.25	15.6	9.15	**2.82**
	TPI-00405-00-A	69.52	96.82	2.78	0.41	ND	ND	ND	ND	ND
	TPI-00406-00-A	73.64	96.01	NA	NA	37.7	ND	ND	ND	ND
	TPI-00407-00-A	65.96	94.89	NA	NA	37.8	ND	ND	ND	ND
	TPI-00408-00-A	67.87	96.18	NA	NA	89.9	18.5	ND	22.5	**1.22**
	TPI-00409-00-A	53.50	92.17	NA	NA	0	ND	ND	ND	ND
	TPI-00410-00-A	59.61	91.67	NA	NA	1.85	ND	ND	ND	ND
	TPI-00411-00-A	70.51	97.43	NA	NA	0.75	ND	ND	ND	ND
	TPI-00412-00-A	81.63	97.98	NA	NA	9.95	ND	ND	ND	ND
	TPI-00413-00-A	50.23	94.78	NA	NA	20.2	ND	ND	ND	ND

Compound Structure	TPI Number	recombinant thioesterase %Inhibition (10μM) TE1	recombinant thioesterase %Inhibition (10μM) TE2	recombinant thioesterase IC_{50} TE1 (μM)	IC_{50} TE2 (μM)	% inhibition of ^{14}C-acetate incorp. PC3 cells	cell survival, MTS assay (IC_{50}) tumor cells PC3	cell survival, MTS assay (IC_{50}) tumor cells DU-145	cell survival, MTS assay (IC_{50}) normal cells FS-4	therapeutic index FS-4/PC3
	TPI-00414-00-A	52.21	86.40	NA	NA	92.4	25	ND	33	**1.32**
	TPI-00415-00-A	79.69	97.46	ND	ND	7.05	ND	ND	ND	ND
	TPI-00416-00-A	64.31	96.49	ND	ND	89.9	25.8	ND	33	**1.28**
	TPI-00417-00-A	74.81	97.85	ND	ND	96.1	13.8	ND	21	**1.52**
	TPI-00418-00-A	65.54	95.92	ND	ND	92.2	31.7	ND	37	**1.17**
	TPI-00419-00-A	66.19	95.79	ND	ND	67.95	32	ND	50	**1.56**
	TPI-00420-00-A	61.96	95.35	ND	ND	68.5	37.5	ND	42	**1.12**
	TPI-00421-00-A	100.00	100.00	1.02	ND	70	17.9	ND	41	**2.29**
	TPI-00422-00-A	22.92	86.76	NA	NA	0	48	ND	>50	ND
	TPI-00423-00-A	79.75	97.99	NA	NA	87.25	20	ND	25	**1.25**
	TPI-00424-00-A	92.59	100.00	NA	NA	0	27	ND	50	**1.85**
	TPI-00425-00-A	0.00	73.61	NA	NA	11.1	ND	ND	ND	ND
	TPI-00426-00-A	48.37	79.24	NA	NA	30.1	ND	ND	ND	ND

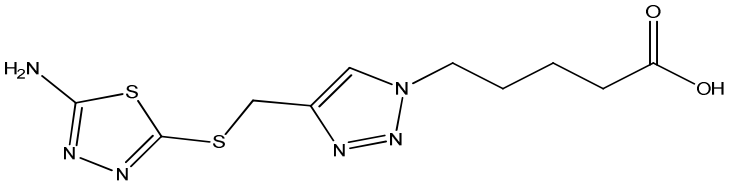
Structure		
 O=S1(CCN(CC2=CN(N=N2)[C@@]3(C[C@@H](C4)C5)C[C@H]5CC4C3)CC1)=O		
 O=S1(CCN(CC2=CN(CC3=CC(C(O)=CO3)=O)N=N2)CC1)=O		
 O=S1(CCN(CC2=CN([C@H]3[C@H](OC)C[C@@H]4[C@H](OC(C5=CC=CC=C5)OC4)[C@@H]3O)N=N2)CC1		
 O=C(/C(S1)=C/C2=C(OCC)C=C(OCO3)C3=C2)N(CC4=CN([C@H]5[C@H](OC)C[C@@H]6[C@H](OC(C7=CC=		



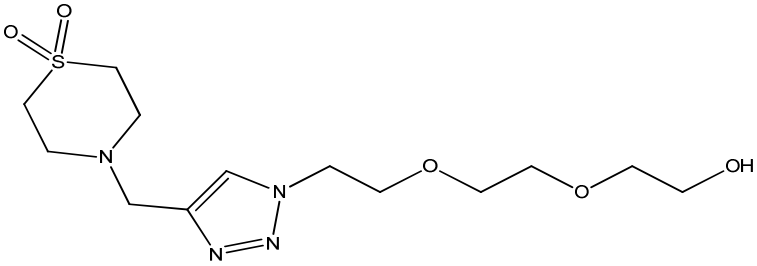
BrC1=CC=C(/C(C#N)=C\C2=C(OCC3=CN([C@@H]4C[C@@H](N5C(NC(C(C)=C5)=O)=O)O[C@H]4CO)N=N3



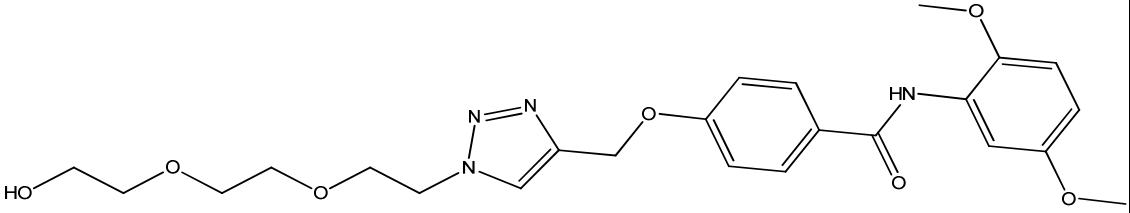
O=C(NC(CC)(C1=CN([C@H]2C[C@H](N3C(NC(C(C)=C3)=O)=O)O[C@@H]2CO)N=N1)CC)NC4=CC(OC)=CC



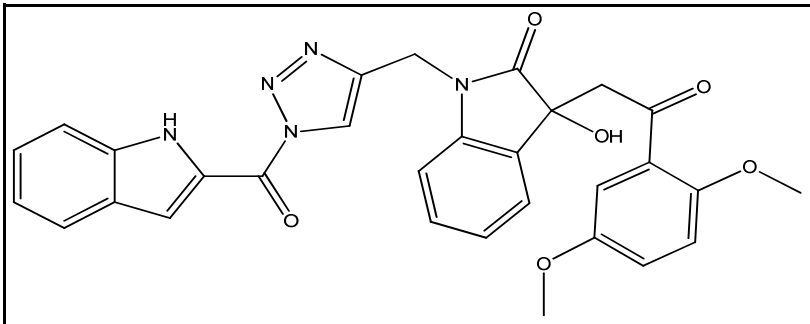
O=C(O)CCCCN1N=NC(CSC2=NN=C(N)S2)=C1



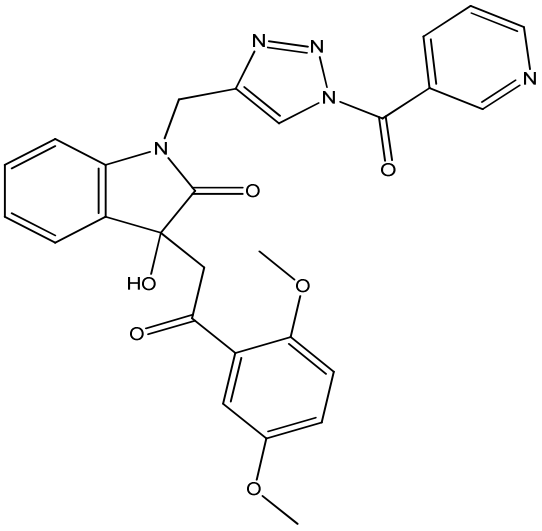
O=S1(CCN(CC2=CN(CCOCCOCCO)N=N2)CC1)=O



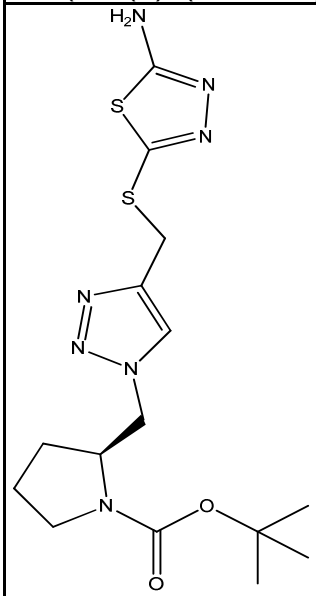
O=C(C1=CC=C(OCC2=CN(CCOCCOCCO)N=N2)C=C1)NC3=CC(OC)=CC=C3OC



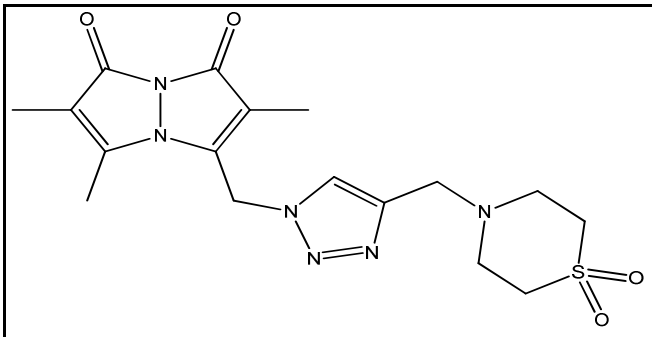
O=C(CC1(O)C(C=CC=C2)=C2N(CC3=CN(C(C4=CC5=CC=CC=C5N4)=O)N=N3)C1=O)C6=CC(OC)=CC=C6O



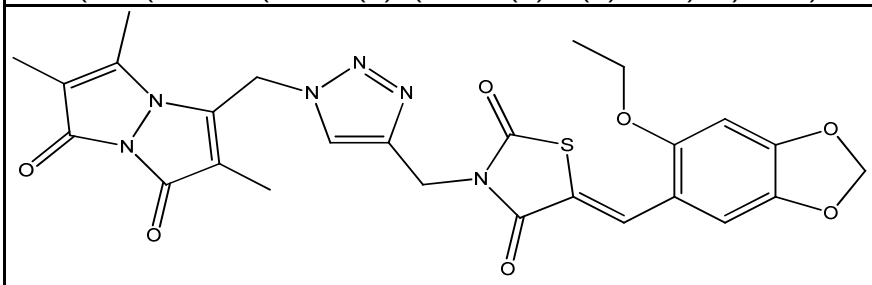
O=C(CC1(O)C(C=CC=C2)=C2N(CC3=CN(C(C4=CC=CN=C4)=O)N=N3)C1=O)C5=CC(OC)=CC=C5OC



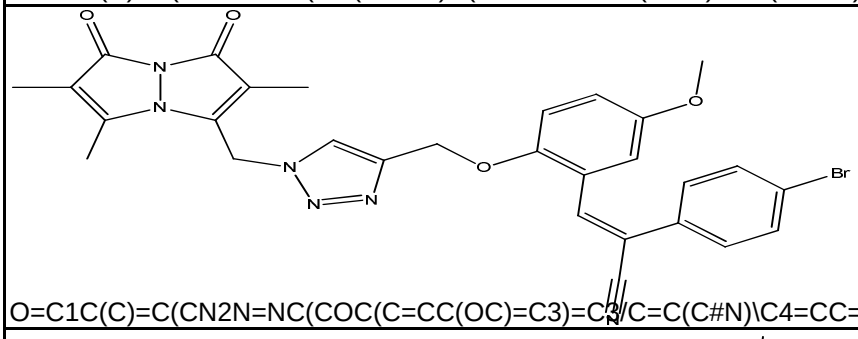
NC1=NN=C(SCC2=CN(C[C@@H]3CCCN3C(OC(C)(C)C)=O)N=N2)S1



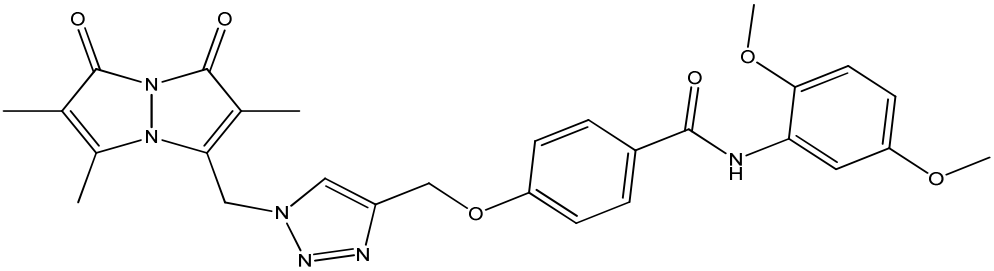
O=S1(CCN(CC2=CN(CC3=C(C)C(N4N3C(C)=C(C)C4=O)=O)N=N2)CC1)=O



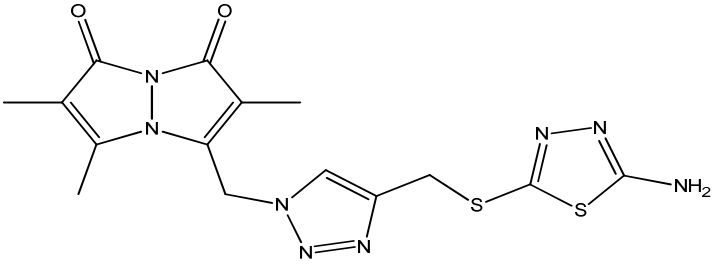
O=C1C(C)=C(CN2N=NC(CN(C/3=O)C(SC3=C/C4=C(OCC)C=C(OCO5)C5=C4)=O)=C2)N6N1C(C(C)=C6C)=O



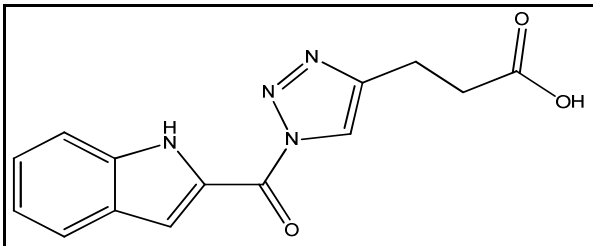
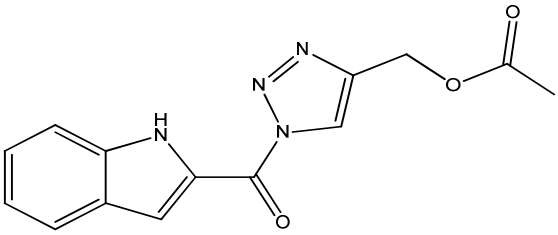
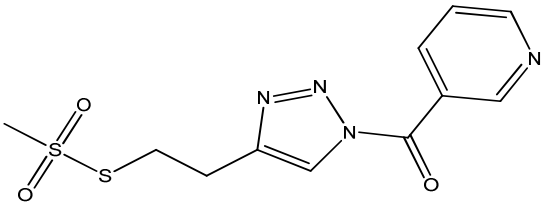
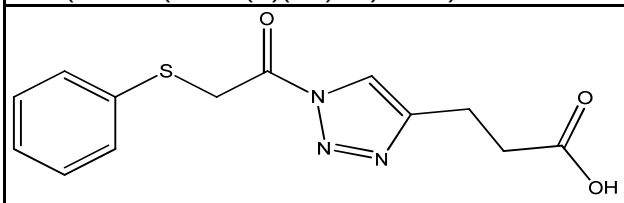
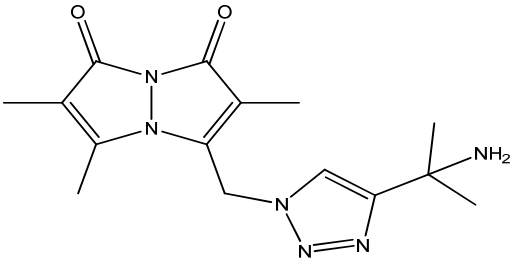
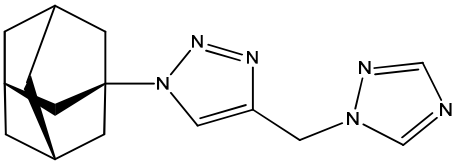
O=C1C(C)=C(CN2N=NC(COC(C=CC(OC)=C3)=C3/C=C(C#N)\C4=CC=C(Br)C=C4)=C2)N5N1C(C(C)=C5C)=C



O=C(C1=CC=C(OCC2=CN(CC3=C(C)C(N4N3C(C)=C(C)C4=O)=O)N=N2)C=C1)NC5=CC(OC)=CC=C5OC



O=C1C(C)=C(CN2N=NC(CSC3=NN=C(N)S3)=C2)N4N1C(C(C)=C4C)=O

 O=C(N1C=C(CCC(O)=O)N=N1)C2=CC3=CC=CC=C3N2		
 O=C(N1C=C(COC(C)=O)N=N1)C2=CC3=CC=CC=C3N2		
 O=C(N1C=C(CCSS(C)(=O)=O)N=N1)C2=CC=CN=C2		
 O=C(N1C=C(CCC(O)=O)N=N1)CSC2=CC=CC=C2		
 O=C1C(C)=C(CN2N=NC(C(C)(N)C)=C2)N3N1C(C(C)=C3C)=O		
 N1(N=NC(CN2N=CN=C2)=C1)[C@@]3(C[C@@H](C4)C5)C[C@H]5CC4C3		